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EVALUATING THE POTENTIAL ANTIHYPERALGESIC EFFECTS OF CURCUMIN ON A LABORATORY MODEL OF NEUROPATHIC PAIN

An Undergraduate Research Scholars Thesis

by

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Submitted to Honors and Undergraduate Research
Texas A&M University
In partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Research Advisor: Dr. Mary Meagher

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Major: University Studies: Honors

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ABSTRACT

Evaluating the Potential Antihyperalgesic Effects of Curcumin on a Laboratory Model of Neuropathic Pain. (May 2014)

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Curcumin is an anti-inflammatory and anti-oxidative, herbal supplement that has been utilized in the animal model to evaluate its potential for anti-inflammatory and anti-nociceptive effects among other benefits. This ongoing pilot study examined whether humans show a similar pattern of benefits after acute consumption of curcumin supplements for seven days. Participants underwent two laboratory visits in which capsaicin-induced spontaneous pain, neurogenic inflammation, primary and secondary hyperalgesia, and heat-pain detection thresholds were measured at baseline and post-treatment. Topical capsaicin mimics neuropathic pain by sensitizing the peripheral and central pain pathway. We hypothesized that curcumin would have anti-inflammatory and anti-hyperalgesic effects that would inhibit the neurogenic inflammatory flare response while also inducing an anti-hyperalgesic effect indicated by reduced ratings of spontaneous pain and stimulus-evoked primary and secondary hyperalgesia. Results have shown a decrease in suprathreshold pain intensity and unpleasantness ratings as well as capsaicin-induced spontaneous pain intensity and unpleasantness ratings. Furthermore, results suggest a marked increase in valence during pain testing during the second visit in addition to increased dominance ratings and decreased arousal. Preliminary results show that curcumin may be an

effective anti-hyperalgesic supplement in a healthy undergraduate population. Ongoing tests will give further insight into curcumin's potential on impacting pain processes.

ACKNOWLEDGEMENTS

I extend immense appreciation to my research mentor, Dr. Mary Meagher, who is the most exceptionally brilliant woman I know. She allowed me to be part of her laboratory early on in my undergraduate experience, and it was monumental for me because she not only provided a great environment for learning and development, but also an environment of support and friendships among the selected members of the laboratory. She guided me throughout the development and execution of my research scholars project, and I cannot thank her enough for giving me the opportunity. I also thank Hans Linsenbardt for helping me immensely with this project as a co-investigator, and he is extremely reliable in all he does; I would not have been able to execute this study without his expertise and his ability to fix any and all issues with Labview. Sophia Dokyoung You taught me and mentored me from the moment I entered the lab, and her meticulousness, extraordinary memory, and optimism has helped me more than I could ever imagine. Rachel Haney made my life so much easier by helping me maintain my composure and by helping me with countless efforts to assist my statistical dilemmas. Fenan Rassu, Brent Furl, Sergiu Albu, and Evan Loehle-Conger helped me work out anything that went wrong, and they have supported me throughout times of confusion and of confidence. They were critical resources for me during write-ups and slip-ups throughout study-preparation. Emma Napoli was a superb research assistant, and I couldn't have kept sane or as organized without her help. Furthermore I acknowledge Sabinsa Corporation for providing our curcumin and placebo materials used in this study. I extend a final thanks to Dr. Duncan Mackenzie and Tammis Sherman for my acceptance into the extraordinary program that University Research Scholars is and for their mentorship in my coursework and Undergraduate Research Ambassadors.

NOMENCLATURE

SAM	Self Assessment Manikin
VAS	Visual Analog Scale
C-ESD	Center for Epidemiologic Studies Depression Scale
DASS	Depression Anxiety Stress Scale

CHAPTER I

INTRODUCTION

In 2010, a survey-based study deduced that chronic pain plagues 30.7% of the United States population, and a majority of those experiencing chronic pain are of lower socioeconomic status and thus less financially prepared to treat their condition (Johannes et al., 2010). Furthermore, since chronic pain is one of the most common reasons for medical visits, solutions are needed to alleviate this common problem as current treatments are not sufficient to manage a condition as complex as pain (Gureje et al., 1998). The healthcare cost attributed to pain in 2010 was between 261 and 300 billion dollars with an additional loss of 229 to 335 billion dollars in costs from lost productivity due to pain (Gaskin and Richard, 2012). This cost is greater than that of heart disease, cancer, or diabetes, and it does not even account for "nursing home residents, children, military personnel, or persons who are incarcerated" (Gaskin and Richard, 2012). Recent research has found that Curcumin, also known as turmeric, an herb used in food spices and herbal medicine, has therapeutic effects due to its anti-inflammatory properties (Arora et al., 1997; Kandhare et al., 2012; Singh, 2007). The anti-inflammatory and antioxidant effects of curcumin may be responsible for its medicinal use against chronic diseases that involve neurodegenerative, cardiovascular, and metabolic diseases (Sandur et al, 2007). Oxidative stress plays a role in the causation of chronic pain, and antioxidants like curcumin reverse these effects by assisting in the elimination of free radicals (Kandhare et al., 2012). Recent studies in animals have shown that curcumin is antihyperalgesic and attenuates levels of inflammatory cytokines, which determine the initiation, severity, and maintenance of pain as well as the inflammation response observed in injured tissue (Kandhare et al., 2012; Yeon et al., 2010). Animal studies

have shown that curcumin attenuates thermal hyperalgesia (the experience of increased sensitivity from a noxious thermal stimulus), mechanical allodynia (pain from an innocuous mechanical stimulus), and capsaicin induced hyperalgesia (Kandhare et al. 2012; Mittal et al, 2009; Tajik et al., 2008; Yeon et al., 2010; Zhao et al., 2011). To our knowledge, this is the first study performed on humans looking at the effects of curcumin on neurogenic inflammation and hyperalgesia. This study will give insights into the effects of curcumin on human pain processes and will serve as a pilot study for further research into its therapeutic effects on chronic pain.

This study's goal is to determine whether curcumin alters measures of peripheral and central sensitization of pain in a human model by comparing differences in the flare response (neurogenic inflammation), thermal pain threshold, suprathreshold pain, capsaicin-induced primary and secondary hyperalgesia, and perception of pain intensity between those taking curcumin and those taking placebo. Primary hyperalgesia involves the experience of increased sensitivity to a noxious stimulus at the site of application, and secondary hyperalgesia involves this experience in tissue surrounding the application site. We hypothesize that participants will have significantly reduced flare and hyperalgesia responses to capsaicin after curcumin intake compared to participants with placebo after seven days.

Curcumin has shown anti-inflammatory and antioxidant effects that may act to decrease neurogenic inflammation and its role in the sensitization of pain. Mounting evidence suggests that curcumin may reduce inflammation and pain by downregulating proinflammatory cytokines' expression, which normally play a major role in the sensitization of pain. Prior research indicates that curcumin downregulates the production of these inflammatory cytokines, chemokines, and

prostaglandins, including interleukin-1 (IL-1), IL-6, Tumor Necrosis Factor-alpha (TNF-alpha), IL-8, MIP-1alpha, NCP-1, and Cox-2 by blocking the activation of the nuclear factor kappa B (NF-κB) transcription pathway. The NF-κB pathway is a key regulator of proinflammatory cytokine release (Aggarwal & Harikumar, 2009). Previous studies indicate that inflammatory cytokines enhance neurogenic flare, the perception of pain, and the area of secondary hyperalgesia, a measure of chronic pain (Kandhare et al., 2012; Sharma, 2006).

In addition to affecting pain through inflammation and oxidants, the structure of curcumin has been shown to inhibit thermal hyperalgesia induced by capsaicin, most likely due to the vanilloid structure shared by capsaicin and curcumin that helps curcumin block the TRPV1 receptor (Gupta et al., 2012; Yeon et al., 2010). Furthermore curcumin inhibits NF-κB transcription of cytokines (Aggarwal & Harikumar, 2009). The current study is designed to further research on curcumin and its therapeutic effects as previous studies involving this herbal supplement have not yet looked at peripheral and central sensitization of pain in a human laboratory model. Peripheral and central sensitization of pain involve an alteration of the properties of neurons in the central nervous system, which results in a change in synaptic efficacy causing enhanced pain sensitivity. Importantly, these processes are known to contribute to the induction and maintenance of chronic pain. Peripheral sensitization occurs only at the site of injury and is caused by inflammation, which reduces nociceptor thresholds. The present experiment aims to determine whether the previous anti-inflammatory and anti-hyperalgesic effects of curcumin observed in animal pain studies will translate to humans. Thus, if curcumin reduces capsaicin-induced laboratory pain using the same methods and measures of chronic pain processes as used in prior animal studies, it may provide a safe and affordable means to prevent or mitigate the

inflammation and hyperalgesia associated with chronic pain conditions such as fibromyalgia or arthritis.

Curcumin is inexpensive, safe in high doses even at 12 grams a day in humans, and is declared as "generally regarded as safe (GRAS)" by the United States FDA (Cheng et al., 2001; Lao et al., 2006; Moreaux et al., 2004). Many clinical trials have involved curcumin and its effect on chronic diseases, and the majority of studies have observed biological responses and symptom reduction (Aggarwal & Sung, 2009; Goel A. Kunnumakkara AB, 2007). This study will compare the effects of curcumin on various properties of pain based on acute consumption of either curcumin or placebo for seven days.

CHAPTER II

METHODS AND MATERIALS

All procedures were approved by the IRB at Texas A&M University, and informed consent was obtained from all participants. As participants received course credits for their participation, they were informed that they could withdraw from the study at any time without forfeiting the credit, and they could instead do extra credit as described in their course syllabus. Data obtained from the subjects were de- identified so only number identified the participants, not name or initials.

2.1 Participants

Undergraduates at Texas A&M University enrolled in Psychology 107 were prescreened to determine eligibility to enroll in the study. Participants of 18 years of age and older who met inclusion criteria were sent an invitation email to participate in the study. The exclusionary criteria included current use of psychoactive drugs, history of vasovagal syncope (fainting), chronic illness, allergy to chili pepper, a skin condition or injury on the forearm, alcohol or drug abuse, use of steroids, ACE-inhibitors, blood thinners, antihistamines, and mood altering medications. Furthermore, participants could not be a smoker, pregnant, nursing, vegetarian, or diabetic. They could not have a BMI of 30 or higher, indicating obesity, consume fish oil, flaxseed, turmeric, or capsaicin regularly, or eat more than two portions of oily fish per week such as salmon or anchovies. Also, they could not have recurrent digestive problems, convulsion disorder, autoimmune or inflammatory diseases, and they could not take any pain, allergy, or recreational drugs within two days prior to the experiment. They could not have acute illness on the day of the experiment or have consumed alcohol within the previous twelve hours. Caffeine

consumption within four hours of the study and regular or recent use of non-steroidal anti-inflammatories also excluded participants. The exclusion criteria are justified to avoid variability and to collect data on overall healthy individuals that do not regularly ingest supplements that could have similar effects as the study supplement, curcumin, and that do not take medications that could have an effect on their pain response. In addition, much of the exclusion criteria involved chronic illness, pain, or obesity in order to exclude participants with possible high amounts of inflammation that could also influence data as curcumin is an anti-oxidant and anti-inflammatory.

Among the 21 subjects, 2 were unable to participate due to their ineligibility upon arrival to the laboratory; 2 participants did not show up to either the first or second visit. Furthermore, some analyses may include fewer than seventeen subjects as a result of equipment failure, but they will be labeled as such. Participants were 17 college students with a mean age of 19.06 years (SD= .66), and 82% were female (see *Table 1* for demographics). The mean BMI of the participants was 22.35 (SD=2.46) ranging from 18 to 28.

***Table 1**: Demographics*

Demographics	**N = 17**
Males	3
Females	14
Right handed	17
Caucasian	14
Asian	2
Latin American	1

2.2 Apparatus and Physiological Recording

Participants were tested in a sound proof room. Blood pressure was collected after questionnaires at the beginning of the experiment using Biopac MP150 equipment (Biopac Systems Inc. Goleta, California). Skin temperature and room temperature were periodically measured to account for major changes.

2.3 Self-Report Data

Participants answered questionnaires during both visits in order to assess psychological states, perception of pain, and general health status. Questionnaires were presented via Qualtrics Survey Software and LabView.

2.3.1 Center for Epidemiologic Studies Depression Scale

The Center for Epidemiologic Studies Depression Scale - CESD (Appendix A) was used during both lab visits as it measures trait depression and anxiety characteristics within the previous week, which has been shown to alter one's pain state (Radloff, L.S., 1991; Rhudy and Meagher, 2000).

2.3.2 The Depression Anxiety Stress Scale

The Depression Anxiety Stress Scale – DASS (Appendix A) was used during both lab visits because it evaluates one's current depression and anxiety state, which can play a role in one's pain state (Lovibond, P. F., and Lovibond, S. H., 1995; Rhudy and Meagher, 2000).

2.4 Self Assessment Manikin (SAM)

The Self Assessment Manikin was used throughout both visits of the study to measure psychophysical experience of pain as it evaluates pleasure, arousal, and dominance, which assists in understanding the subject's perception of pain intensity and unpleasantness across various stimuli (Bradley and Lang, 1994). Higher ratings of valence correspond to a more positive mood with a lower rating associated with a negative mood. Similarly, a higher rating in arousal corresponds to a more physiologically aroused state, and a higher numerical rating in dominance indicates a greater sense of control of the situation. The subjects underwent SAM training during the both visits, and SAM was completed before, after, and during pain testing throughout both visits.

2.5 Capsaicin

Each visit involved the application of 1 milliliter of Zostrix capsaicin lotion with a .075% concentration. It was applied to the non-dominant volar forearm for thirty minutes following five minutes of heat stimulation using the Medoc Advance Thermal Stimulator (Medoc Ltd. Ramat Yishai, Israel). Capsaicin was used to induce a flare response, spontaneous pain and primary hyperalgesia. The Capsaicin used in this experiment is the active ingredient found in peppers like jalapenos and habanero peppers. It is a naturally derived ingredient that is safe for consumption. Capsaicin is also used in over-the-counter products and creams used to treat pain and muscle soreness, including brand names such as Capsin or Zostrix. Capsaicin has been used for years as a standardized method in studies to mimic aspects of pain analogous to chronic pain conditions; it has been used extensively in behavioral and psychological studies.

2.6 Curcumin

This study is predominantly evaluating the anti-hyperalgesic effects of curcumin, also known as turmeric, which is an herb used in food spices and herbal medicine with therapeutic effects due to its anti-inflammatory and anti-oxidative nature (Arora et al., 1997; Kandhare et al., 2012; Singh, 2007). Curcumin is available over-the counter, inexpensive, safe in high doses even at 12 grams a day in humans, and is declared as "generally regarded as safe (GRAS)" by the United States FDA (Cheng et al., 2001; Lao et al., 2006; Moreaux et al, 2004). Curcumin capsules (1000 mg each) used in the study were provided by Sabinsa Corporation (Curcumin C3 Complex ®, Sabinsa Corporation Payson, UT). Participants were informed that they may receive a placebo or natural supplement in a double-blind process so that neither the experimenter nor the participant would be aware of the material assigned. Furthermore, participants were given instructions to take two tablets every evening and morning between 8 and 10 PM and 8 and 10 AM every day including the day of the first visit. Subjects were instructed to respond to reminder texts with a "y" after each time prompted to take the supplement once it was consumed. Reminder texts were scheduled using Google Voice and an SMS scheduling extension provided by Google Chrome. All contact information was stored in a secure place that only the co-investigators had access to.

2.7 Pain Ratings

Participants used a visual analog scale (VAS) presented on the computer using LabView version 8.0 software (National Instruments, Austin, TX) to make ratings of pain intensity and unpleasantness throughout the experiment. The scale ranges from 0 to 10 with verbal descriptors as anchors beginning with 0 (no pain) to 10 (most intense pain imaginable) and 0 (not at all

unpleasant) to 10 (most unpleasantness imaginable). This scale was used during von Frey training, long thermal stimulation, capsaicin application, and testing for secondary hyperalgesia.

2.8 Von Frey Testing

Von Frey filaments were used to assess secondary hyperalgesia. These filaments are composed of flexible nylon and are organized according to thickness. As they increase in thickness and gram units as indicated on each brush, the pressure of their application increases. The von Frey must be held perpendicular to the target, and it is pressed onto the skin until it bends. We used a 26 gram filament immediately after the flare image is taken after the thirty minutes of capsaicin. The covering and capsaicin lotion were gently removed without rubbing the lotion into the skin more or spreading it outside of the site, and the von Frey was pressed onto each spoke of each radian of the grid beginning from the outside and moving inward. Each participant also was trained to make ratings with the von Frey during the first visit, during which only two radians were drawn onto the dominant arm, and two different von Frey sized filaments (26g, and 300g) were applied to the arm for each participant. Subjects rated the pain intensity for each spoke on the VAS, and were instructed to differentiate between pain and pressure.

2.9 Thermal Threshold Pain Testing

Participants underwent assessment of thermal pain thresholds using Medoc Advance Thermal Stimulator (Medoc Ltd. Ramat Yishai, Israel). They were asked to place the thenar eminence of the non-dominant hand on a three by three centimeter thermode. The warm thermode began at 35 degrees Celsius and increased at a rate of 0.5 degrees Celsius per second until participants

clicked a mouse at the first sensation of pain. The thermode had a cutoff temperature of 51 degrees in order to prevent injury. This procedure was described during informed consent, and instructions were given beforehand. Each participant had one practice trial, and three additional trials from which the average was taken to determine his or her pain threshold. Furthermore, participants repeated this method after capsaicin application during which threshold was determined on the application site immediately following von Frey testing. The thermode was covered with plastic wrap, and it was placed onto the site for four trials.

2.10 Heat-Capsaicin Application

To begin spontaneous pain testing with capsaicin application, participants placed a warm thermode (Medoc Ltd. Ramat Yishai, Israel) onto the application site, which was heated at forty-five degrees Celsius for five minutes to prime the area. This served as a long thermal stimulation test (LTS) as the participant rated his or her pain intensity every minute on the VAS. Immediately after the five minutes, the capsaicin solution was applied and covered for thirty minutes during which the participant made spontaneous pain intensity and unpleasantness ratings every three minutes along with SAM. This heat-capsaicin method has been used many times in previous studies, and it is utilized to initiate stable cutaneous sensitization resulting from the heat and cream so that we may use a lesser solution (Dirks et al., 2003; Petersen et al., 2001; Petersen and Rowbotham, 1999).

2.11 Data Analysis

Paired t-tests were used to compare within groups between visit 1 and visit 2 for measures involving two variables. Repeated measures ANOVAs were performed in order to compare within group effects on multiple variables.

2.11.1 Handling Missing Data Values

Some values were missing due to equipment failure throughout the tests. As a result, the group means were utilized to replace any missing values that occurred during the suprathreshold pain 5 minutes of 45° heat, thirty minutes of capsaicin-induced spontaneous pain, and von Frey secondary hyperalgesia mapping.

2.12 Procedure

Participants entered the laboratory and were instructed to take off jewelry on their wrists and fingers. They washed their hands and forearms, and eligibility criteria were checked again to ensure no recent ingestion of antihistamines, caffeine, alcohol, pain medicine or recreational drugs as well as no acute illness that day. Body Mass Index (BMI) was also assessed using an electronic scale and measuring tape attached to the wall. Participants then sat down in the testing room, listened to an audio track, and read a physical copy of the informed consent. Subjects then filled out questionnaires presented via Qualtrics Survey Software, which are listed in section 2.3. The subjects were then prompted to undergo SAM training and SAM baseline was assessed thereafter as described in section 2.4. After questionnaires, blood pressure measures were taken; if blood pressure were abnormal, the subjects were ineligible to continue with the study.

The participants were then instructed how to indicate thermal pain thresholds explained in section 2.9, and they participated in four heat- threshold trials. They were then given instructions on how to rate their pain using the VAS, and they were asked to recall the last time they cut their finger and to rate the intensity and unpleasantness of that experience on the VAS to ensure the instructions were understood. Next, the von Frey map was drawn in its entirety on the non-dominant arm with a washable marker during both visits. The first visit also included a grid with only two radians drawn on the dominant arm for training described in section 2.8. Skin temperature and room temperature were collected before the five minutes of heat began on the non-dominant volar forearm. During the five minutes of steady heat, participants made SAM and spontaneous pain ratings on the VAS each minute.

Measurements of skin temperature were then collected again before applying capsaicin lotion to the application site. The subjects were instructed to make VAS ratings of pain intensity and unpleasantness every three minutes as prompted by the computer. The experimenter left the room after the participant made the first rating and instructions were understood. They were monitored via a web-camera in the room, of which they were informed. Subjects could also contact the experimenter using a walkie-talkie left in the room with them. After the thirty minutes of capsaicin, the covering was removed. Skin temperature and room temperature were measured, and a flare image of blood flow was taken using a moorLDI2 Laser Doppler Imager (MoorLDI2-IR, Moor Instruments, Devon, United Kingdom). Von Frey testing for area of secondary hyperalgesia took place immediately after the flare image (section 2.8). Thereafter, participants underwent thermal thresholds utilizing the Medoc once again; however, this time

applied to the application site. To prevent capsaicin residue from getting onto the thermode, a plastic wrap was wrapped around it before being placed onto the forearm (section 2.9).

Finally, all of the remaining capsaicin lotion was removed with vegetable oil, and the participants thoroughly washed their forearm. The area of application was covered in case of any remaining lotion. During the first visit, participants were given their supplement along with instructions for the next seven days, which is described in section 2.6. At the second visit, after curcumin consumption, participants went through the same procedures as the first visit except for the von Frey training.

CHAPTER III

RESULTS

3.1 Psychological Characteristics

The CES-D and DASS self-report questionnaires were administered at the beginning of each visit to evaluate depression for the former and depression, stress, and anxiety for the latter. Participants did not significantly differ between visits in their perceived stress ($t_{(14)} = .68$, $p = .50$), anxiety scores ($t_{(14)} = .70$, $p = .50$), or depression scores ($t_{(14)} = .57$, $p = .58$) in the DASS. Furthermore, the groups did not differ in depression scores evaluated by the CES-D ($t_{(15)} = .62$, $p = .55$).

3.2 Heat Pain Detection Thresholds

Paired t-tests were used to compare heat pain detection thresholds before and after curcumin consumption. There was no significant difference between visit 1 ($M = 43$, $SEM = .55$) and visit 2 ($M = 43$, $SEM = .49$), ($t_{(16)} = .87$, $p = .40$), as seen in *Figure 1*.

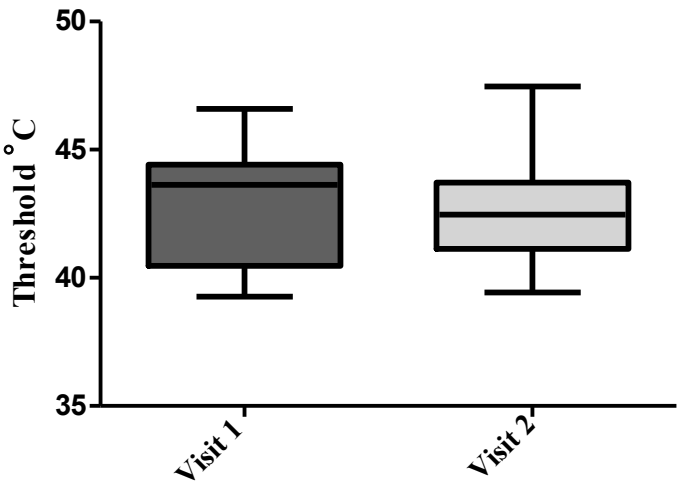

Figure 1*:* Comparison of heat pain detection thresholds of which there is no significant difference $p > .1$.

3.3 Suprathreshold Spontaneous Pain Ratings

A two-way repeated measures ANOVA revealed a significant difference between the baseline suprathreshold pain intensity ratings and post-curcumin ratings $F_{(1,32)} = 11.80$, $p < .01$, which can be seen in *Figure 2*. There was also a significant effect of time, $F_{(4,256)} = 2.97$ $p < .05$; however, there was no significant interaction of time and visit $p = .76$. Similarly, a two-way repeated measures ANOVA showed a significant effect of visit on unpleasantness ratings using the VAS during the 5 minutes of heat (*Figure 3*), $F_{(1,32)} = 8.78$, $p < .01$; however, there was no effect of time $p = .22$, nor was there an interaction of time and visit $p = .67$.

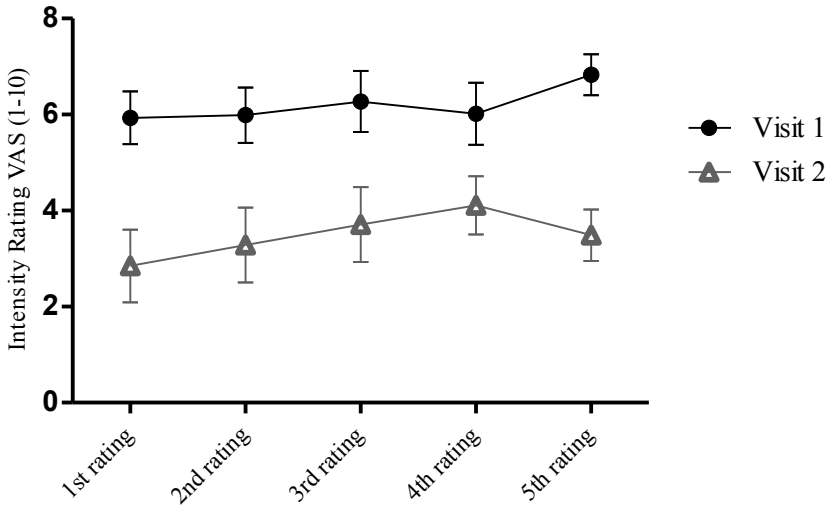

Figure 2: Suprathreshold spontaneous pain intensity ratings over 5 minute period of 45° Celsius heat between the baseline visit 1 and visit 2 after consuming curcumin for 7 days $p < .01$. Ratings were made on a Visual Analog Scale.

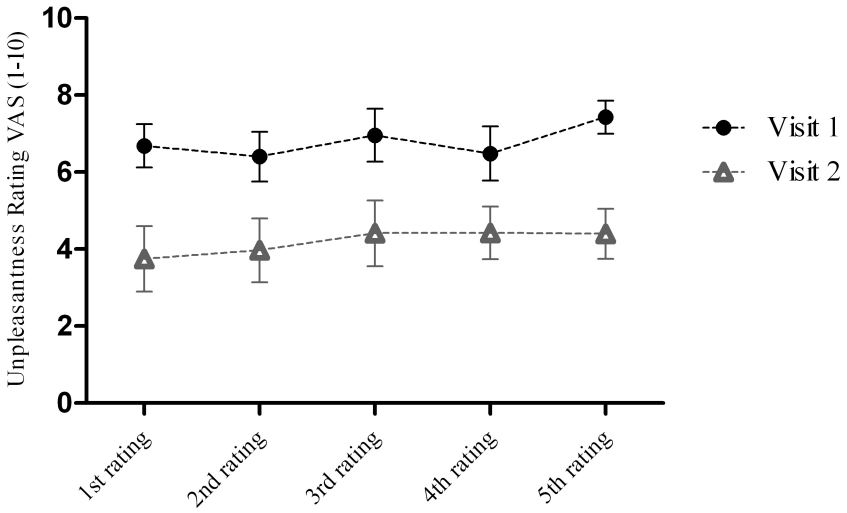

Figure 3: Suprathreshold spontaneous pain unpleasantness ratings over 5 minute period of 45° Celsius heat between the baseline visit 1 and visit 2 after consuming curcumin for 7 days $p < .01$. Ratings were made on a Visual Analog Scale.

3.4 Affective Response to Pain

A two-way repeated measures ANOVA found that there was a marked difference in valence between the two visits, $F_{(1,80)} = 88.10$, $p < .01$, seen in *Figure 4*. There was not a significant effect of time $p = .81$, nor was there an interaction effect $p = .64$. Based on the results of another two-way repeated measures ANOVA, there was also a significant effect of visit on arousal (*Figure 5*), $F_{(1,80)} = 38.98$, $p < .01$, but there was no main effect of time $p = .90$, nor was there an interaction $p = .60$. Finally, a two-way repeated measures ANOVA found a significant effect of visit on dominance, $F_{(1,80)} = 36.90$, $p < .01$, see *Figure 5*, but it did not find a marked effect of time $p = .91$. There was also no significant interaction of time and visit $p = .48$.

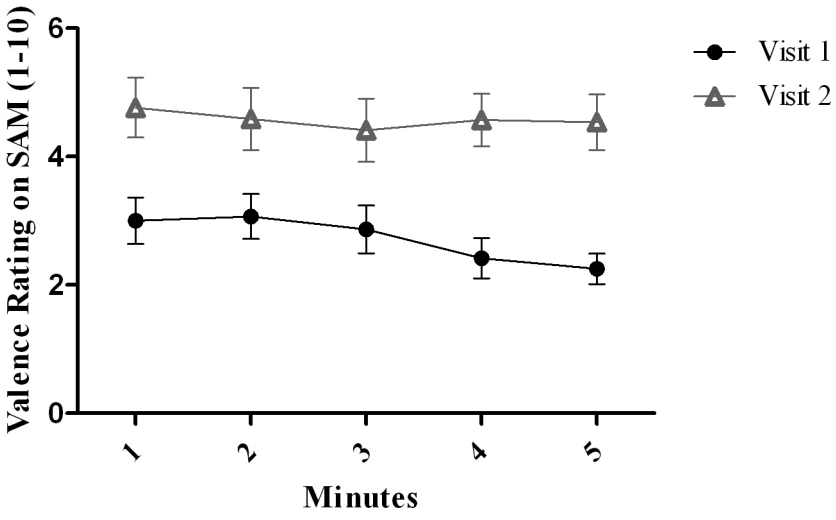

Figure 4: SAM ratings of valence before and after curcumin consumption. Higher ratings correspond with decreased negative affect, $p < .01$. Ratings were made each minute for five minutes of 45° Celsius heat.

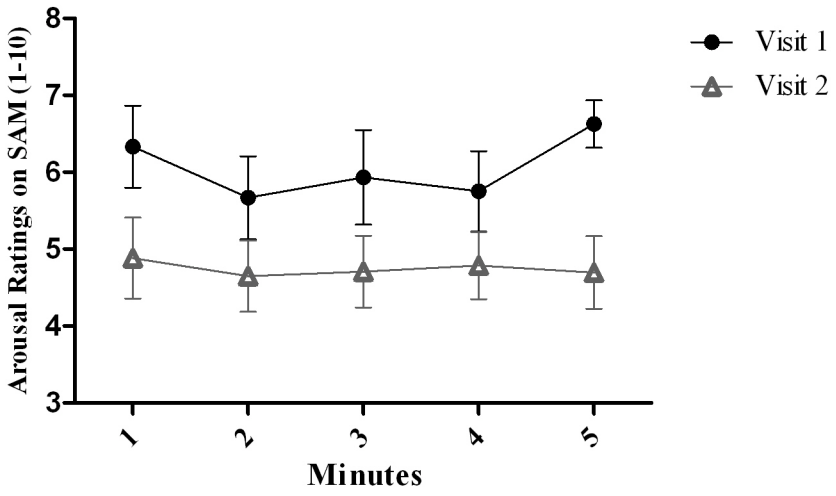

Figure 5: SAM ratings of arousal after curcumin consumption, which significantly differed, $p <$ .01. Higher ratings correspond with increased arousal. Ratings were made each minute for five minutes of 45° Celsius heat.

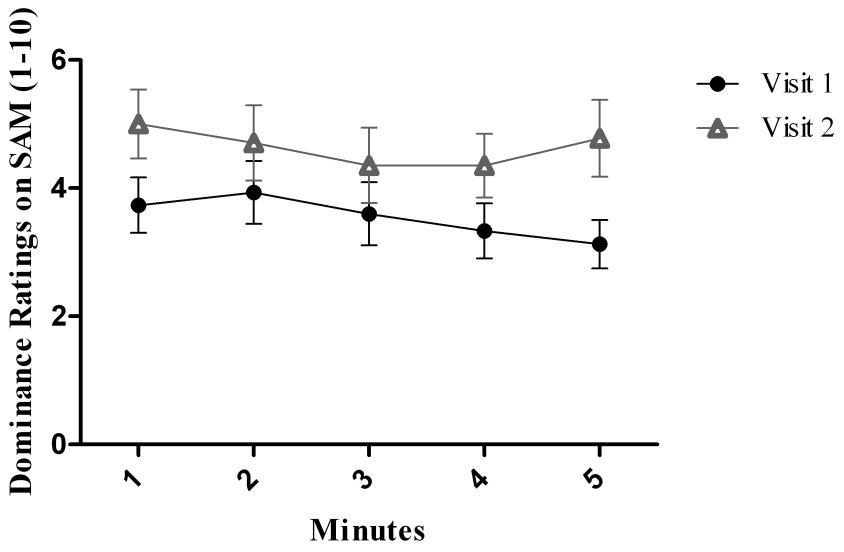

Figure 6: SAM ratings of dominance before and after curcumin consumption, which significantly differed as ratings of dominance increased during the second visit indicating a greater sense of control, $p < .01$. Ratings were made each minute for five minutes of 45° Celsius heat.

3.5 Capsaicin-Induced Spontaneous Pain Ratings

A two-way repeated measures ANOVA revealed a significant effect of visit on spontaneous pain intensity ratings made by participants every three minutes during the thirty minutes of capsaicin application following a five minute heat stimulus, $F_{(9,288)} = 17.34$, $p < .01$. Ratings were significantly decreased during the second visit compared to the first visit, see *Figure 7*. Furthermore, there was no significant effect of time, $p = .09$, nor was there an interaction of time and visit $p = .25$. In addition, a two-way repeated measures ANOVA showed a significant effect of visit on spontaneous unpleasantness ratings, $F_{(9,288)} = 20.88$, $p < .01$, such that ratings were

significantly decreased after curcumin consumption, see *Figure 8*. There was not a significant effect of time $p = .17$, nor was there a marked interaction between visit and time, $p = .15$.

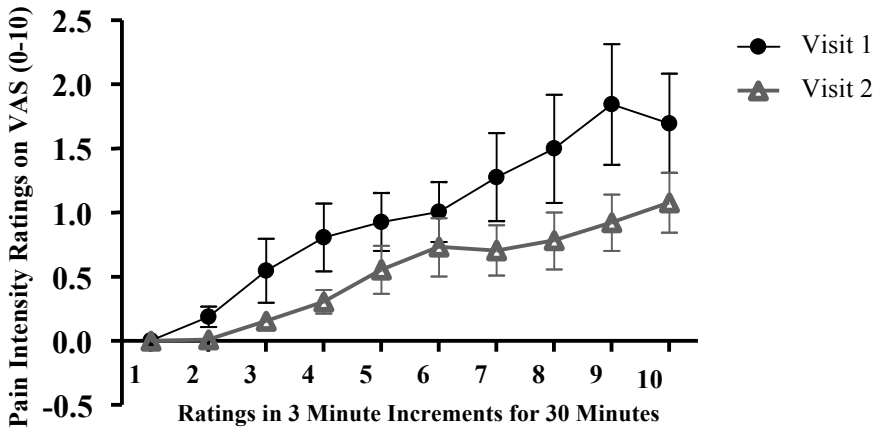

Figure 7: Pain intensity ratings on a VAS during the thirty minutes of capsaicin application, $p < .01$. Ratings were made every three minutes.

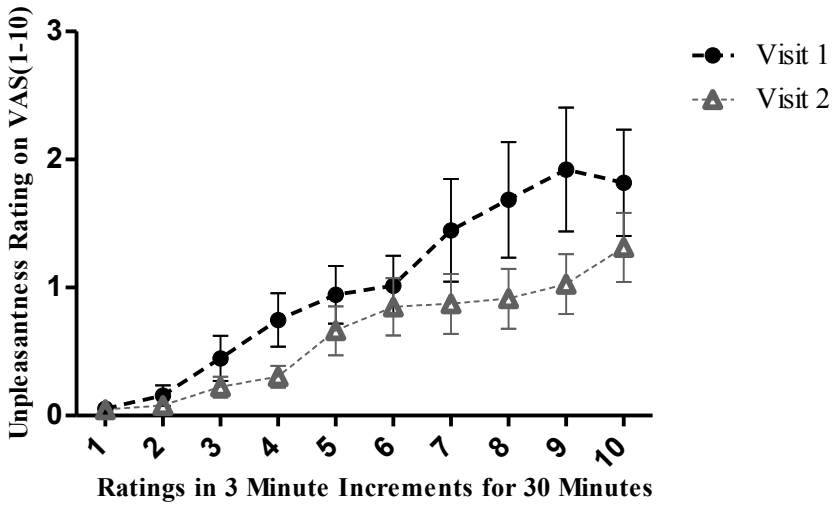

Figure 8 Unpleasantness ratings on a VAS during the thirty minutes of capsaicin application, $p <$ .01.

3.6 Area of Secondary Hyperalgesia

A paired t-test found no significant difference between the area of secondary hyperalgesia during the first baseline visit, $M = 45$ cm^2, SEM = 9.8 cm^2, and the second visit after curcumin intervention, $M = 55$ cm^2, SEM = 11 cm^2, ($t_{(16)} = 1.3$, $p = .21$), as seen in *Figure 9*.

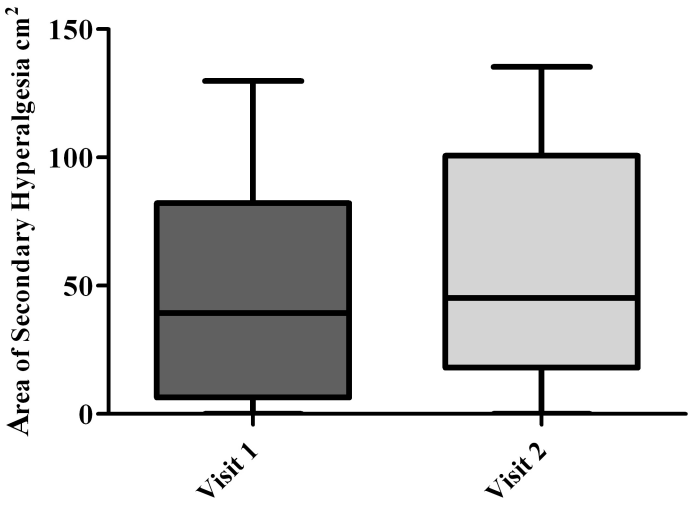

Figure 9: Area of secondary hyperalgesia from von Frey mapping, $p = .21$.

3.7 Neurogenic Inflammation

After performing a paired t-test, no significant difference in flare was observed between the first visit, $M = 44$, $SEM = 3.5$, and the second visit after curcumin consumption, $M = 41$, $SEM = 3.9$, ($t_{(16)} = 1.3$, $p = .22$), as seen in Figure 10.

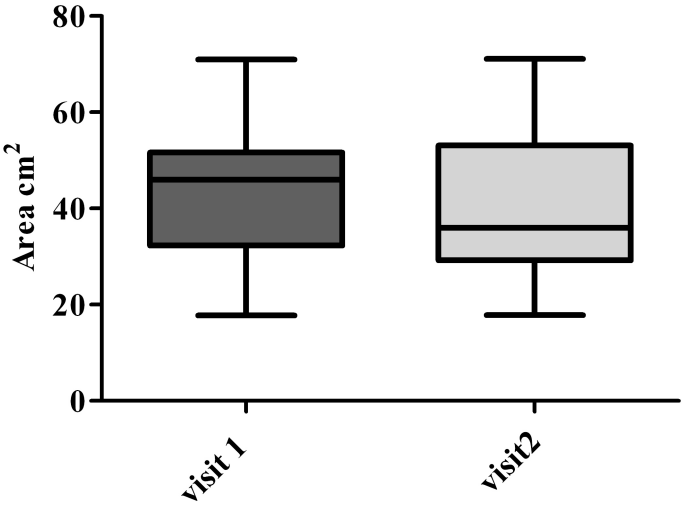

Figure 10: Comparison of flare area observed between visit 1 and visit 2, $p = .22$.

3.8 Adherence to Supplement Assignment

Participants were asked to return their remaining supplement tablets upon the second laboratory visit of the study. 15 participants out of 17 brought back their remaining tablets or empty containers, and their percentage of assigned supplement consumed based on this method is illustrated in *Figure 11*, $M = 84.52\%$, $SD = 18.29$.

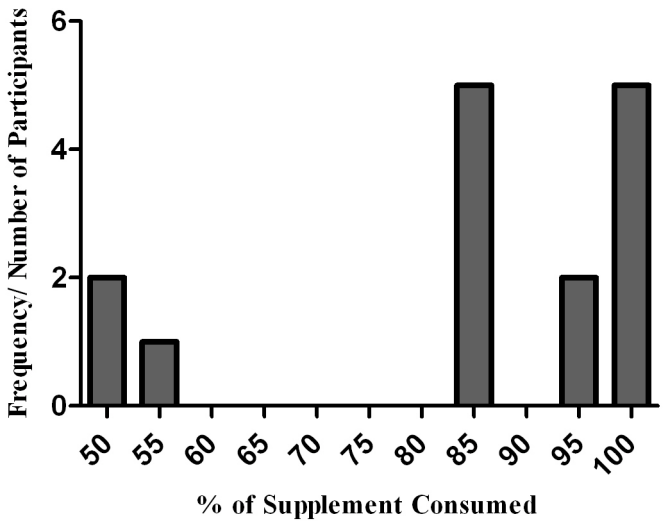

Figure 11: Percent of consumed supplement for the 15 participants that returned their containers.

CHAPTER IV

DISCUSSION

The purpose of this study was to test the potential anti-hyperalgesic effects of a curcumin supplement on pain processes within a healthy population. Results provide preliminary support for several of our initial hypotheses. As expected, curcumin significantly reduced pain intensity and unpleasantness ratings during suprathreshold thermal stimulation at 45 degrees Celsius, a pain stimulus that activates C-fibers, but it had no effect on threshold stimulation that activates A- delta fibers. Furthermore, we observed a marked effect of curcumin on capsaicin-induced thermal hyperalgesia, which was reflected by reduced pain intensity and unpleasantness ratings during the thirty- minute capsaicin application period. Capsaicin-induced pain mimics many of the symptoms of neuropathic pain and engages many of the same underlying mechanisms. Specifically, it also activates C-fibers via the transient receptor potential vanilloid 1 (TRPV1) receptor and elicits a similar burning and tingling effect experienced in those with chronic pain. Curcumin's antihyperalgesic effect on capsaicin-induced neurogenic pain is in line with previous animal studies our methods emulated (Yeon et al., 2010). Thus, the effects observed in prior animal studies appear to generalize to humans.

Furthermore, our participants reported a significant decrease in negative affect and arousal as well as an increase in dominance or locus of control during the suprathreshold pain test. This is interesting but not surprising, as other studies have found similar effects of curcumin on mood due to its nature to downregulate inflammatory cytokines (Anand et al., 2008; Kulkarni, et al., 2009). The mood elevating effects of curcumin have been attributed to its ability to downregulate proinflammatory cytokines. Reduced negative affect and arousal could also be related to reduced

pain sensitivity since Strand et al. (2007) found that arthritis patients reported minimized pain during weeks they also reported more positive affect. Further investigation could provide insight into the relative contribution of curcumin on the relationship between affect and pain.

Although we did not observe an effect of curcumin on area of secondary hyperalgesia and neurogenic inflammation (flare), there was a slight trend of decreased flare between visits. The unexpected lack of robust findings in neurogenic inflammation thus far is possibly due to the small sample size or to individual differences in stressful life events or adherence to the curcumin regimen. These factors may have also contributed to the negative effect on secondary hyperalgesia. Alternatively, the area of secondary hyperalgesia may not be as sensitive a measure as mechanical allodynia previously used in animal studies of curcumin's effects on neuropathic pain (Jeon et al., 2013). To evaluate this possibility, future studies will incorporate measures of mechanical allodynia and hyperalgesia.

Spontaneous pain and primary hyperalgesia observed in response to heat stimulation at the application site reflects the sensitization of the primary afferent nociceptors, which in turn contribute to the process of central sensitization of spinal cord dorsal horn neurons and pain pathways in the brain. In contrast, secondary hyperalgesia in response to mechanical stimulation in the area surrounding capsaicin application reflects the underlying process of central sensitization. Because most clinical pain syndromes involve both peripheral and central sensitization during the development of chronic pain, the anti-hyperalgesic effects of curcumin on the capsaicin pain test in healthy humans are likely to predict the therapeutic effects on clinical pain.

There were no significant differences in heat pain thresholds before or after capsaicin application, which is counter to our hypothesis because the post-capsaicin thresholds are also a measure of thermal primary hyperalgesia, which animal studies have seen a difference in the tail-withdrawal test after sensitization from reserpine injection (Arora, et al., 2011). However, the lack of difference in thresholds is in line with a separate study's evaluation of rats that showed no effect of curcumin on withdrawal response latencies without capsaicin (Yeon, et al., 2010). Yeon has shown that curcumin acts by antagonizing TRPV1 receptors. TRPV1 receptors are activated by temperatures greater than 43 °C (109 °F) and by capsaicin. Whether an effect of curcumin on heat pain threshold is observed or not may depend on the heat stimulus ramp speed and peak heating temperature of the skin. A rapid ramp speed and high peak temperature may allow the heat stimulus to activate TRPV1 receptors before eliciting a reflexive withdrawal response (e.g., Arora et al. 2011). Moreover, other evidence suggests that the occurrence of visual or auditory cues predicting the onset of the heat stimulus may allow subjects to make an avoidance responses to the heat before it has reached pain threshold (King et al. 1997), prior to the activation of TRPV1 receptors (e.g., Yeon et al 2010). Because cues are readily available in Medoc threshold testing in human subjects, it may have allowed subjects to make avoidance responses thereby preventing TRPVI activation. To resolve this issue, future studies will need to examine whether increasing stimulus ramp speed during threshold testing and shielding subjects from cues of heat onset yield a different pattern of results.

Important limitations to consider in this study are, as stated before, the mental and physical health of our sample. Age, health conditions, chronic pain, BMI, and diet were all considered in

this study resulting in a healthy set of individuals with low inflammatory tone. The small sample size is also a limitation with a study that involves many measures and the possible variability due to the stress of testing and pre-existing individual differences. Because the participants were college students, their lives fluctuated in regard to stress as a result of tests, sleep, and diet, which can introduce individual variation that is not present in animal studies where one can easily control the environmental histories and genetic backgrounds of the subjects. Another limitation was ensuring that the college students receiving credits for their psychology course were taking their supplements. They were sent reminder texts, given instructions verbally and on paper, and asked to bring their remaining tablets back, but it is still difficult to be certain of accountability. In addition, although our results are significant, we hope that the findings will be more robust after future incorporation of a placebo comparison group. By administering both placebo and curcumin to separate groups, we will be able to compare within and between groups to eliminate the possibility of an effect of experience in the laboratory on pain perception differences.

To our knowledge, this is the first study evaluating curcumin's anti-hyperalgesic potential on humans in a laboratory setting. After acquiring more data from a larger sample size, we will examine whether the amount of supplement consumed may correspond to the resulting pain experience in a dose-dependent manner. Furthermore, these findings support the concept that pain and neurogenic inflammation are regulated by many independent processes, and it brings forth, even more so, the need for continued pain-related research. Should curcumin continue to induce significant effects on laboratory pain in healthy subjects, the next step will be to

investigate curcumin's effects in a clinical population with chronic pain and neurogenic inflammation.

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